DATA EVALUATION RECORD

STUDY 5

CHEM 112600	Prohexadione calcium	§162-3
CAS No. 127277-53-6		
FORMULATION--00--ACTIVE INGREDIENT		

STUDY ID 44457786

Singh, M. 1996. Anaerobic aquatic metabolism of ^{14}C-BAS 125 W. BASF Report No.: M9503. BASF Reg. Document No.: 96/5083. Unpublished study performed by BASF Corporation, Research Triangle Park, NC, and Agvise Laboratories, Northwood, ND; and submitted by BASF Corporation, Research Triangle Park, NC.

DIRECT REVIEW TIME = 53 Hours

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CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. This study is scientifically valid and provides upgradable supplemental information that:

   A. Prohexadione calcium, at a nominal concentration of 0.19 ppm (μg/g soil), degraded with a EFED calculated half-life of 117 days in flooded loamy sand soil incubated anaerobically in darkness at 25 ± 1 °C for up to 365 days.

   B. The major degradate was despropionyl. It was present initially at time 0 in the water phase at 2.2% of the applied radioactivity and increased to a maximum of 53.3% by 180 days posttreatment. In the soil phase the degradate despropionyl was initially 1.9% of the applied radioactivity at 52 days and increased to a maximum of 6.7% by 365 days.

2. This study does not satisfies the guideline requirement for an anaerobic aquatic metabolism study for the following reasons:

   A. The study author did not provide any data to show that the anaerobic conditions were maintained (see The Reviewers Comments 2)

   B. Details of the HPLC conditions were not supplied (see The Reviewers Comments 3).

3. In order to satisfy the data requirement, the study author needs to submit the following information:

   A. data from monitoring anaerobic conditions via indicator strips throughout the incubation, and

   B. details of the HPLC conditions.

   upon which this study may be upgraded to meeting the guidelines.

METHODOLOGY

Samples (30 g) of sieved (2 mm) loamy sand soil (87% sand, 7% silt, 6% clay, 0.7% organic matter, pH 5.3, CEC 4.1 meq/100 g; Appendix 1, p. 71) collected from Holly Springs, NC, were placed in centrifuge tubes and flooded with 30 mL of pre-purged (with nitrogen) well water (pH 9.0, conductivity 0.18 mmhos, hardness 17 mg/L as $CaCO_3$, total dissolved solids 154 ppm; Appendix 1, p. 73); the soil:water ratio was 1:1 (w:v; p. 12), and N_2 was purged through the soil/water system for 5 min. The samples were placed in glass incubation towers equipped with an inlet valve for introducing nitrogen, and an outlet valve attached to two CO_2 traps (2.0 *N* NaOH) and an organic volatile trap (ethylene glycol) in succession (p. 14; Figure 4 and Figure 5,

p. 49). The soil/water systems were purged with nitrogen and pre-incubated in the incubation towers (nitrogen atmosphere) in darkness at 25 ± 1 °C for 62 days to achieve anaerobic conditions; anaerobic conditions were determined by anaerobic indicator strips placed inside the incubation towers (pp. 12, 13). Following the pre-incubation period, the soil/water systems were treated (by injection under the water surface) with cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium (BAS 125 W, calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate, radiochemical purity 98.9%, specific activity 15.3 mCi/mmol; pp. 9, 10), dissolved in acetonitrile and hydrochloric acid, at nominal concentrations of 0.19 ppm (µg/g soil) and 1.9 ppm (µg/g soil; pp. 13, 14; Appendix 2, p. 76). High dose samples were only used for the identification of the parent compound and its degradates. The treated tubes were purged with nitrogen and incubated anaerobically (nitrogen atmosphere) in sealed glass towers in darkness at 25 ± 1 °C for up to 365 days. Triplicate samples were removed for analysis at 0, 21, 52, 87, 137, 180, 245, and 365 days posttreatment; one replicate at each sampling interval was stored in a freezer as a reserve sample. Volatile traps were analyzed by LSC at all sampling intervals (p. 15).

Figure 6 presents the flow chart of the analytical method for the water phase. Each soil/water sample was centrifuged and the supernatant was decanted (p. 15; Figure 6, p. 51). The residual soil was washed twice with distilled water. The three supernatants were combined and analyzed for total radioactivity by LSC. The samples from 0 and 21 days posttreatment were concentrated by solid phase extraction (SPE; SAX SPE-Empore disc) and the disc was eluted with water followed by acetonitrile (p. 16). Total radioactivity in the eluate was determined by LSC. To quantify the radiolabeled residues that were retained in the disc, 1 M trifluoroacetic acid (TFA):acetonitrile (ACN; volume ratio unspecified) was passed through the disc; the eluate was collected and total radioactivity determined by LSC. The TFA:ACN eluate was concentrated, rediluted with ACN, and analyzed by HPLC (conditions not specified). Eluate fractions were analyzed by LSC. The disc was analyzed for total radioactivity by LSC following combustion. Supernatants from all samples removed after 21 days posttreatment were lyophilized and the residue was transferred to a flask with acetone:ACN:1 *N* H_2SO_4 (4.5:4.5:1.0, v:v:v). The samples were sonicated, centrifuged, and analyzed by LSC and HPLC (conditions not specified; pp. 16, 17).

The flow chart of the analytical method for the soil samples is presented in Figure 13. Soil samples were extracted three times by sonicating and vortexing with acetone:ACN:1 *N* H_2SO_4 (4.5:4.5:1, v:v:v) or acetone:1 *N* H_2SO_4 (9:1, v:v; 0 and 21 day samples only; pp. 15, 18; Figure 13, p. 58). Combined extracts were analyzed for total radioactivity by LSC. To determine nonextractable residues, subsamples of dried, post-extracted soil were analyzed for total radioactivity by LSC following combustion. Extracts from 0 and 21 day posttreatment samples were concentrated on a rotary evaporator, rediluted with water, and partitioned three times using ethyl acetate (p. 19). Combined ethyl acetate and combined water phases were analyzed for total radioactivity by LSC. The ethyl acetate phase was concentrated, transferred to a flask and rediluted with ACN. The solutions were sonicated, centrifuged, and analyzed by LSC and HPLC (conditions not specified); eluent fractions were analyzed by LSC. Extracts from samples removed after 21 days posttreatment were concentrated on a rotary evaporator, transferred to a flask and rediluted with distilled water, sonicated, centrifuged, and analyzed by LSC and HPLC (conditions not specified; p. 19).

To confirm the identity of the parent compound (W4; BAS 125 W) in the water phase, samples collected at 21 days posttreatment were concentrated by SPE (SAX SPE-Empore disc) and the disc was eluted with ACN:TFA (unspecified volume ratio; pp. 17, 18). The ACN:TFA eluate was concentrated on a rotary evaporator and redissolved in distilled water; the pH was adjusted to 3.0 with NH_4OH. The solution was applied to a preconditioned SPE cartridge (ENV_+). The cartridge was eluted with distilled water followed by methanol. The methanol eluent was collected, concentrated and analyzed by HPLC (YMC-PAK ODS-AQ column) using a mobile phase gradient of ACN:water (both with 0.1% TFA; 5:95 to 100:0, v:v) with radioactive flow detection (pp. 11, 17). The sample was further analyzed by two-dimensional TLC using silica gel plates developed perpendicularly with THF:n-hexane:HCO_2H (83:15:2, v:v:v) followed by ACN:HCO_2H (98:2, v:v).

To confirm the identity of the parent compound (S3; BAS 125 W) in the soil phase, the ethyl acetate phase from the day 21 soil extract was applied to a preconditioned SPE cartridge SAX Empore disc and eluted successively with EtOAc, ACN, and 1 M formic acid:ACN (unspecified volume ratio; p. 20); eluates were analyzed by LSC. The ACN eluate was concentrated and analyzed by HPLC (Hamilton PRP-1 column) using a mobile phase gradient of ACN:water (both with 0.1% TFA; 2:98 to 100:0, v:v) with radioactive flow detection (p. 21); samples were co-chromatographed with nonradiolabeled parent compound which was visualized with UV (unspecified wavelength) light. A second aliquot of the ethyl acetate phase from the day 21 soil extract was analyzed by two-dimensional TLC as described previously. The sample was co-chromatographed with the nonradiolabeled parent compound.

To confirm the identity of the degradate despropionyl (W1; BW9054-5376) in the water phase, day 319 samples (high treatment rate only) were lyophilized (p. 17). The residual material was redissolved in acetone:ACN:formic acid (4.5:4.5:1, v:v:v) and analyzed by HPLC using YMC-PAK ODS-AQ and Hamilton PRP-1 columns as described previously. Samples were co-chromatographed with the nonradiolabeled reference standard of despropionyl which was visualized with UV (unspecified wavelength) light.

To confirm the identity of the degradate despropionyl (S1; BW9054-5376) in the soil phase, soil from 319 days posttreatment was extracted three times with acetone:ACN:1 $N\ H_2SO_4$ (4.5:4.5:1, v:v:v) and concentrated on a rotary evaporator (p. 20). An aliquot of the solution was applied to a preconditioned SPE cartridge and eluted successively with distilled water, dichloromethane, and MeOH; eluates were analyzed by LSC. The MeOH eluate was concentrated and analyzed by HPLC using YMC-PAK ODS-AQ and Hamilton PRP-1 columns as described previously; samples were co-chromatographed with the nonradiolabeled reference standard of despropionyl which was visualized with UV light.

Samples of post-extracted soils from 245 and 365 days posttreatment were further extracted three times by sonicating with 1 *N* NaOH and centrifuged (pp. 21, 22). The supernatants were decanted, combined, and aliquots neutralized with 1 *N* HCl were analyzed by LSC. The NaOH extract was acidified to pH 2, applied to a preconditioned SAX SPE cartridge, and successively eluted with distilled water, ACN, MeOH, and ACN:Et_3N (50:0.5, v:v). Combined eluates were concentrated, redissolved in ACN, and analyzed by HPLC using YMC-PAK ODS-AQ and Hamilton PRP-1 columns as described previously; samples were co-chromatographed with the nonradiolabeled reference standard of despropionyl.

Storage stability was assessed by analyzing samples collected at 0 and 245 days posttreatment before and after storage below 0 °C for 91 and 99 days, respectively. Table 10 (p. 45; see Comment #3) contains the data.

THE STUDY AUTHOR'S DATA SUMMARY

Cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium (radiochemical purity 98.6%), at a nominal concentration of 0.19 ppm (μg/g soil), degraded with a registrant-calculated half-life of 86.5 days (r^2 = 0.95; p. 31; Appendix 5, pp. 94, 95) in flooded loamy sand soil incubated anaerobically in darkness at 25 ± 1 °C for up to 365 days.

Data, reported as percentages of the applied, represent percentages of the nominal application. In the whole test system, the parent compound was initially 91.4% of the applied radioactivity. It was 67.7-69.4% of the applied at 21-52 days, decreased to 44.8% by 87 days and was 5.9-7.7% at 245-365 days posttreatment (Table 7, p. 42). In the water phase, the parent compound (W4) was initially 82.3% of the applied radioactivity, decreased to 49.5% of the applied by 21 days posttreatment, was 58.3% of the applied at 52 days posttreatment, and was 3.0-3.9% at 245-365 days posttreatment (Table 3, p. 38). The major degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (W1; despropionyl, BW9054-5376) was initially 2.2% of the applied radioactivity in the water phase at day 0, increased to a maximum of 53.3% of the applied by 180 days posttreatment, and was 33.4% at 365 days posttreatment. An unidentified minor degradate (designated as W2) was initially 5.0% of the applied radioactivity at 87 days posttreatment, increased to a maximum of 6.2% of the applied by 137 days posttreatment, and was 1.9% at 365 days posttreatment. An unidentified minor degradate (designated as W3) was initially 1.3% of the applied radioactivity at 52 days posttreatment and increased to a maximum of 5.1% of the applied by 365 days posttreatment.

In the soil phase, the parent compound (S3) was initially 9.1% of the applied radioactivity, increased to a maximum of 20.0% of the applied by 21 days posttreatment, and decreased with variability to 2.0% by 365 days posttreatment (Table 6, p. 41). The degradate despropionyl was initially present at 1.9% of the applied radioactivity at 52 days posttreatment and increased to a maximum of 6.7% of the applied by 365 days posttreatment. An unidentified minor degradate (designated as S2) was initially 0.52% of the applied radioactivity at 180 days posttreatment and increased to a maximum of 3.2% of the applied radioactivity by 365 days posttreatment.

Nonextractable [^{14}C]residues were initially 0.64% of the applied radioactivity at day 0, increased to 12.5% by 87 days and were a maximum of 32.9% of the applied radioactivity at 365 days posttreatment (Table 4, p. 39). After further extraction with NaOH, an additional 17.7% and 22.9% of the applied radioactivity was extracted from the 245 and 365 day posttreatment samples, respectively (Table 9, p. 44). All radioactivity was identified as despropionyl (p. 32; Figure 20, p. 65). Evolved $^{14}CO_2$ accounted for a maximum of 3.2% of the applied radioactivity at 137 days posttreatment and varied from 0.28-1.4% from 180 to 365 days (Table 7, p. 42); [^{14}C]organic volatiles were not detected (p. 26). The distribution ratio of [^{14}C]residues between the soil and water fractions was not reported, however, the majority of the [^{14}C]residues was observed in the water phase from 0 to 245 days (Table 1, p. 36). The reviewer-calculated soil:water residue distribution ratio was 1:8.5 at day 0, 1:1.6 to 1:5 from 21 to 245 days posttreatment and was 1:1 at 365 days posttreatment.

Material balances were 89.3-102.8% of the applied radioactivity with no clear pattern of loss (Table 1, p. 36).

THE REVIEWERS' COMMENTS

1. Cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium, at a nominal concentration of 0.19 ppm (µg/g soil), degraded with an EFED calculated half-life of 117 days (R^2= 0.95) in flooded loamy sand soil incubated anaerobically in darkness at 25 ± 1 °C for up to 365 days. The major degradate was despropionyl. In the water phase the degradate was initially present at 2.2% of the applied radioactivity at time zero and increased to a maximum of 53.3% by 180 days posttreatment. In the soil phase it was initially 1.9% of the applied radioactivity at 52 days posttreatment, and increased to a maximum of 6.7% by 365 days. There were two unidentified minor degradates which did not exceed 6% of the applied at any time. Nonextractable [^{14}C]residues were initially 0.64% of the applied radioactivity at time 0 and increased to a maximum of 32.9% of the applied radioactivity at 365 days posttreatment. After further extraction with NaOH, an additional 17.7% out of 26.42% and 22.9% out of 32.87% of the applied radioactivity was extracted from the 245 and 365 day samples, respectively. All radioactivity was identified as despropionyl. Evolved $^{14}CO_2$ accounted for a maximum of 3.2% of the applied radioactivity at 137 days. [^{14}C]organic volatiles were below lower limits of quantitation.

2. The study author stated that anaerobic conditions were monitored by anaerobic indicator strips placed inside the incubation tower, however, the measured data were not reported. The agency is requesting that the registrant verified that the aerobic conditions were maintained in the test system. Submission of the indicator strip data are needed for this

conformation. In future studies the conformation of anaerobic conditions should be completed using redox potential, dissolved oxygen content and pH of the test systems.

3. The conditions of the HPLC analyses initially used to analyze all samples were not specified. The conditions of the HPLC analyses used to confirm compound identities were reported; however, it could not be determined whether these conditions were also used for the initial analyses. In addition, it could not be determined whether samples of the water phase were co-chromatographed with the nonradiolabeled reference standard of the parent compound.

4. Method detection limits (MDL) and limits of quantitation (LOQ) were not reported for either LSC, HPLC, or TLC analyses of the prohexadione calcium and its degradates.

Prohexadione Calcium

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Pages 8 through 31 are not included.

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